proteins form transient and time-dependent interactions that are central to the function of this oscillator.

1151-Pos Board B43

NMR Structure and Dynamics of the Response Regulator Sma0114 from Sinorhizobium Meliloti

Sarah Sheftic, Andrei Alexandrescu.
University of Connecticut, Storrs, CT, USA.

Receiver domains control intracellular responses triggered by signal transduction in bacterial two-component systems. Here, we report the solution NMR structure and dynamics of Sma0114 from the bacterium *Sinorhizobium meliloti*, the first such characterization of a receiver domain from the HWE-kinase family of two-component systems. The structure of Sma0114 adopts a prototypical α_5/β_5 Rossman-fold but has features that set it apart from other receiver domains. The fourth β-strand of Sma0114 houses a PFxFATGY sequence motif, common to many HWE-kinase-associated receiver domains. This sequence motif in Sma0114 may substitute for the conserved Y-T coupling mechanism, which propagates conformational transitions in the 455 (α4-β5-α5) faces of receiver domains, to prime them for binding downstream effectors once they become activated by phosphorylation. In addition, Sma0114 lacks the fourth α-helix of the consensus 455 face and ^{15}N relaxation data show that it is replaced by a segment that is flexible on the ps-ns timescale. Secondary structure prediction of Sma0114 and other HWE-kinase-associated receiver domains suggests that the absence of helix α4 may be a conserved property of this family. In spite of these differences, Sma0114 has a conserved active site, binds divalent metal ions such as Mg^{2+} and Ca^{2+} that are required for phosphorylation, and exhibits μs-ms active site dynamics similar to other receiver domains. Taken together, our results suggest that Sma0114 has a conserved active site but differs from typical receiver domains in the structure of the 455 face that is used to effect signal transduction following activation.

1152-Pos Board B44

Molecular Dynamics Simulations Support the use of Methyl Generalized Order Parameters in the "Entropy Meter"

Vignesh Kasinath, Kim A. Sharp, Joshua A. Wand.
University of Pennsylvania, Philadelphia, PA, USA.

Conformational entropy is a potentially important thermodynamic parameter contributing to protein function. Unfortunately, conformational entropy has been difficult to measure experimentally. We have been working towards using measures of fast internal motion as a proxy for conformational entropy. Implementation of this approach has been impeded by the apparent need to employ a model-dependent interpretation of the obtained generalized order parameters. We have recently proposed that the quantitative relationship between generalized order parameters and conformational entropy could be determined empirically and effectively create an 'entropy meter' (Marlow et al. Nat. Chem. Biol. 6, 353). This approach has initially employed methyl symmetry axis order parameters that can be obtained relatively easily. The approach then rests on the coupling between motion of the methyl bearing side chains and the rest of the amino acids being sufficient to report on the whole protein. Here, we examine the generality of this assumption using molecular dynamics simulations of a number of proteins. The MD simulations performed with the NAMD suite showed excellent agreement between the calculated and experimental side chain order parameters for all the proteins, a marked improvement from previous studies. We find a general correlation between the internal dynamics of protein and the protein conformational entropy. The calculated protein conformational entropy showed excellent linear correlation to both the calculated NMR order parameter of methyl groups as well as the experimentally measured NMR methyl order parameters. Supported by NIH grant GM102447 and a grant from the Mathers Foundation.

1153-Pos Board B45

Mapping Dynamic Structure along Calmodulin-Target Binding Interfaces using Vibrational Labels

Rebecca B. Wai, James B. Torain, Shannon R. Dalton, Casey H. Londergan.
Haverford College, Haverford, PA, USA.

Calmodulin's highly modular tertiary structure leads to interesting dynamics in its conformational distribution that are difficult to document using conventional methods. A cyanylated cysteine vibrational probe on single site cysteine variants of calmodulin can be used to determine local aspects of calmodulin's dynamic structure around the probe group when bound to different partners. Several new single-cysteine mutants of calmodulin were expressed and purified for the purpose of labeling with cyanylated cysteine and examining differences in its structure around the chosen label sites. Infrared spectra of labeled mutants in the apo, calcium-saturated, and bound states with a variety of calmodulin binding partners will be reported to demonstrate the ability of cyanylated cysteine to map dynamic and modular calmodulin-target binding interfaces.

1154-Pos Board B46

Lower Protein Stability does not Necessarily Increase Local Dynamics

Levi J. McClelland, Sean M. Seagraves, Bruce E. Bowler.
University of Montana, Missoula, MT, USA.

Overall protein stability is thought to have an important impact on the local dynamics modulating enzyme function. In order to better understand the effects of overall stability on local dynamics in mitochondrial cytochrome *c*, we test the effect of a destabilizing Leu85Ala mutation on the dynamics of the tier 0 alkaline conformational transition involving conformational changes on the ms timescale. Elucidating further understanding into protein dynamics has significant relevance to health, as alternative protein conformers are associated with many human diseases. The alkaline conformational transition replaces the Met80 ligand on the heme with a lysine residue from Ω-loop D, the heme crevice loop, consisting of residues 71-85. Residues 67 to 87 are the most conserved portion of the sequence of mitochondrial cytochrome *c*, suggesting this region is of prime importance for function. Mutations to Ω-loop D affect the stability of the heme crevice directly, modulating the pK_{app} of the alkaline transition. Two variants of yeast iso-1-cytochrome *c*, WT*/L85A and WT*/K73H/L85A, were over-expressed in *E. coli* and purified for these studies. Guanidine-HCl unfolding monitored by circular dichroism and pH titrations at 695 nm, respectively, were used to study the thermodynamics of global and local unfolding of these variants. Dynamics of the alkaline transition were measured by pH-jump stopped-flow methods. Contrary to the expectation that dynamics around the heme crevice would be faster for the less stable WT*/K73H/L85A variant, they were similar to those for a variant without the L85A mutation. In fact, below pH 7, the dynamics of the WT*/K73H/L85A variant were slower. Gated electron transfer techniques using bis(2,2',2''-terpyridine)cobalt(II) as a reducing reagent were implemented to measure the heme crevice dynamics for the WT*/K73H/L85A variant.

1155-Pos Board B47

Folding and Stability of Helical Bundle Proteins from Coarse-Grained Models

Abhijeet Kapoor, Alex Travesset.
Iowa State University, AMES, IA, USA.

We develop a coarse-grained model where solvent is considered implicitly, electrostatics is included as short-range interactions and side-chains are coarse-grained to a single bead. The model depends on three main parameters: hydrophobic, electrostatic and side-chain hydrogen bond strength. The parameters are determined by considering three levels of approximations and characterizing the folding for three selected proteins (training set). Nine additional proteins (containing up to 126 residues) as well as mutated versions (test set) are folded with the given parameters. In all folding simulations, the initial state is a random coil configuration. Besides the native state, some proteins fold into an additional state differing in the topology (structure of the helical bundle). We discuss the stability of the native states, and compare the dynamics of our model to all atom molecular dynamics simulations as well as some general properties on the interactions governing folding dynamics.

1156-Pos Board B48

Interfering with the Host-Pathogen Interaction of Bordetella Pertussis

Edithe Selwa[1], Elodie Laine[2], Luca Maragliano[3], Alexandre Chenal[1], Giovanni Ciccotti[4], Daniel Ladant[1], **Thérèse E. Malliavin**[1].
[1]Institut Pasteur, Paris, France, [2]Université Pierre et Marie Curie, Paris, France, [3]Istituto Italiano di Tecnologia, Genoa, Italy, [4]University of Roma ''La Sapienza'' and University College Dublin, Roma, Dublin, Italy.

The adenyl cyclases EF and CyaA play an important role into the virulence of the agent of whopping cough (B.pertussis) and of the agent of anthrax (B.anthracis). Indeed, their interaction with calmodulin (CaM), leads to cAMP overproduction, disorganizing the signaling network into the host cell. The numerous structures of EF recently allowed some of us to analyze dynamics and energetics of EF-CaM (1-2, 4, 6) and to discover a new family of EF inhibitors (5).

The analysis of the interaction between CaM and the catalytic domain (AC) of CyaA was more difficult to handle, as only the X-ray crystallographic structure of the complex of AC with the C terminal lobe of calmodulin (C-CaM) was know. Qualitative information was available on the conformations of isolated AC, revealing a less elongated shape than in the complex with C-CaM.

Molecular dynamics simulations (7) predict three residues, R338, N347 and D360, to stabilize the AC/CaM interaction. Furthermore, their experimental mutations show significant decrease of the AC affinity for CaM, involving these residues in long-range allosteric communication between CaM and the AC catalytic site. Advanced molecular dynamics simulations, based on the TAMD approach(3), were used to investigate conformational landscape of the isolated AC and to propose compacted AC structures.